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85. (Amended) The method as in any of claims 1, 28, or 40, wherein methylation is in both alleles.

REMARKS

A. Regarding the Amendments

The present invention provides methods for detecting hepatic cell proliferative disorders by detecting methylated CpG-containing glutathione-S-transferase (GST) nucleic acid in a hepatic specimen or biological fluid, wherein a methylated GST nucleic acid is indicative of a hepatic cell proliferative disorder. The present invention provides for the first time the ability to detect hepatic cell proliferative disorders by detecting methylated CpG-containing GST nucleic acid. Accordingly, the present invention is useful in detecting and treating a variety of conditions associated with inappropriate hepatic cell proliferation, e.g., cancer.

By the present communication, claims 1, 3, 4, 6, 8, 11, 16, 18, 20, 27, 39, 41, 42, 51, 84, and 85 have been amended to more particularly define Applicants' invention and to address minor informalities regarding a typographical error and claim dependency. Thus, no new matter is introduced by these amendments. Claims 1-51, 84, and 85 are pending.

B. Rejection Under 35 U.S.C. § 112, Second Paragraph

The rejection of claims 1-51, 84, and 85 under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention, is respectfully traversed. With specific reference to the phrase "hepatic cell proliferative disorder", it is respectfully submitted that this term is clear and unambiguous as it is used in claim 1. Those skilled in the art would readily acknowledge that this phrase encompasses any condition wherein hepatic cells are growing in a manner that is atypical as compared to "normal" hepatic cells.. Thus, the Examiner's assertion

that it is allegedly unclear as to whether the subject phrase refers to either hepatic cancers or cysts, is respectfully submitted to be unfounded.

With specific reference to the term "glutathione-S-transferase nucleic acid" (GST nucleic acid), it is respectfully submitted that this term is clear and unambiguous as it is used in claims 1, 2, and 40. Those skilled in the art would readily acknowledge that this term, as used throughout the specification, encompasses any GST nucleic acid. Thus, the Examiner's assertion that it is unclear whether "GST nucleic acid" refers to only nucleic acid or to a chimera comprising GST polypeptide is respectfully submitted to be unfounded.

With specific reference to the phrase "the start site", it is respectfully submitted that this term is clear and unambiguous as it is used in claim 4. Indeed, those skilled in the art would readily understand that the subject phrase refers to the transcriptional start site in the nucleic acid of GST. Nevertheless, to reduce the issues and expedite prosecution, claims 3 and 4 have been amended to explicitly recite "transcriptional start site".

With specific reference to the phrase "methylated nucleic acid", it is respectfully submitted that this term is clear and unambiguous as it is used in claim 6. Those skilled in the art would readily understand that the subject phrase refers to methylated CpG-containing GST nucleic acid. Nevertheless, to reduce the issues and expedite prosecution, claim 6 has been amended to explicitly recite "methylated CpG-containing GST nucleic acid".

With specific reference to the phrases "said amplifying step" and "the amplifying step", as these phrases are used in claims 6 and 7, respectively, Applicants respectfully disagree with the Examiner's assertion that no antecedent basis exists for the subject phrases. Claim 6 clearly recites an amplifying step (see claim 6, line 3). Thus, contrary to the Examiner's assertion, it is respectfully submitted that sufficient antecedent basis exists for the phrases "said amplifying step" and "the amplifying step".

With specific reference to the phrase "have a sequence as set forth in SEQ. ID. NO:...", it is respectfully submitted that this phrase is clear and unambiguous as it is used in claim 8. Those skilled in the art would readily understand that the oligonucleotide primers recited in claim 8 are selected from the group consisting of SEQ. ID. NO: 7, 8, 9, 10, 11, 12, and 13. Nevertheless, to reduce the issues and expedite prosecution, claim 8 has been amended using the transitional phrase "selected from the group consisting of".

With specific reference to the phrase "CpG-containing nucleic acid", it is respectfully submitted that this phrase is clear and unambiguous as it is used in claim 11. Those skilled in the art would readily understand that the subject phrase refers to the CpG-containing GST nucleic acid recited in claim 1. Nevertheless, to reduce the issues and expedite prosecution, claim 11 has been amended to explicitly recite "CpG-containing GST nucleic acid".

With specific reference to claim 14, the Examiner's assertion that claim 14 is indefinite since it is allegedly unclear as to where in the method of claim 6 the step recited in claim 14 is carried out, is respectfully submitted to be irrelevant. It is well established that there is no chronological order implied in the steps of a method claim. Claim 14 further defines the method of claim 6 by simply reciting an additional step. Accordingly, it is respectfully submitted that claim 14 is clear and unambiguous as written.

With specific reference to the phrase "the GST", as this phrase is used in claims 16, 18, 20, 27, and 51, it is respectfully submitted that this rejection is rendered moot by amendment to these claims herein. In addition, with specific reference to the phrase "GST target nucleic acid", as this phrase is used in claim 16, this rejection is rendered moot by amendment to this claim herein.

With specific reference to the phrases "GST DNA" and "GST RNA", it is respectfully submitted that these phrases are clear and unambiguous as they are used in claims 16 and 28.

Those skilled in the art would readily acknowledge that these terms, as used throughout the specification, encompasses any GST DNA or GST RNA containing CpG islands. Thus, the Examiner's assertion that it is unclear whether "GST DNA" or "GST RNA" refers to only nucleic acid encoding GST or to a chimera comprising GST polypeptide is respectfully submitted to be immaterial.

With specific reference to claims 27, 39, and 51, it is respectfully submitted that these claims are clear and unambiguous as written. Those skilled in the art would readily understand that the claims refer to the methylation status of GST nucleic acid compared to the methylation status of GST nucleic acid in normal hepatic tissue. Nevertheless, to reduce the issues and expedite prosecution, claims 27, 39, and 51 have been amended to more particularly define this aspect of Applicants' invention.

With specific reference to the phrase "the nucleic acid", it is respectfully submitted that this term is clear and unambiguous as used in claims 41 and 42. Those skilled in the art would readily understand that the subject phrase refers to GST nucleic acid. Nevertheless, to reduce the issues and expedite prosecution, claims 41 and 42 have been amended to explicitly recite "GST nucleic acid".

For all of the reasons set forth above, it is respectfully submitted that the rejection of claims 1-51, 84, and 85 under 35 U.S.C. § 112, second paragraph is not properly applied. Accordingly, reconsideration and withdrawal of the rejection are respectfully requested.

C. Rejection Under 35 U.S.C. 112, First Paragraph

The rejection of claims 1-51, 84, and 85 under 35 U.S.C. § 112, first paragraph, as allegedly containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to

make and/or use the invention, is respectfully traversed. Applicants respectfully disagree with the Examiner's assertion that the specification allegedly does not teach one skilled in the art how to make and/or use the invention commensurate in scope with the claims.

It is well-established that the claims of a patent application are presumptively enabled when the application is filed. Thus, the burden of demonstrating that the entire breadth and scope of the claims is allegedly not enabled falls on the Examiner. In this case, the Examiner has provided no evidence to call into question the enablement of the claims. Accordingly, for all of the following reasons, it is respectfully submitted that the Examiner has not met the burden of demonstrating non-enablement.

Applicants respectfully disagree with the Examiner's assertion (see Office Action mailed April 23, 2002, page 8, lines 1-5) that the specification allegedly provides no guidance that any hepatic cell proliferative disorder can be detected by detecting a methylated CpG-containing glutathione-S-transferase (GST) nucleic acid. Indeed, the Examiner's assertion that only hepatocellular carcinoma can be detected according to the methods of the present invention is respectfully submitted to be erroneous. Contrary to the Examiner's assertion, those skilled in the art recognize that a direct correlation exists between the presence of methylated CpG-containing GST nucleic acid in a subject and hepatic cell proliferative disorders. For example, as set forth throughout the specification (see, e.g., page 4, lines 8-11; page 6, lines 1-3; page 7, lines 1-9) human liver carcinogenesis proceeds via accumulation of "CpG island" hypermethylation of GST nucleic acid. Thus, it is clear that any hepatic cell proliferative disorder can indeed be detected by detecting a methylated CpG-containing GST nucleic acid. Accordingly, it is respectfully submitted that the entire scope and breadth of the claims (with specific reference to the detection of any hepatic cell proliferative disorder) is enabled.

In efforts to support the particular assertion that the specification allegedly provides no guidance that any hepatic cell proliferative disorder can be detected by detecting a methylated

CpG-containing glutathione-S-transferase (GST) nucleic acid, the Examiner cites Herman, et. al. (U.S. Patent No. 6,017,704) wherein it is disclosed that human genes are known to be methylated. The Examiner therefore concludes that those skilled in the art would allegedly not be able to detect a hepatic cell proliferative disorder based solely on detection of methylated CpG-containing GST nucleic acid (see Office Action mailed April 23, 2002, page 9, lines 1-9). This conclusion is respectfully submitted to be in error. While it is known that several biological functions are attributed to controlled methylation of bases in DNA (such as, e.g., protection of DNA from digestion by cognate restriction enzymes), the present invention provides methods for detecting a methylated CpG-containing GST nucleic acid in a hepatic specimen or biological fluid wherein a methylated GST nucleic acid is indicative of a hepatic cell proliferative disorder. This type of methylation, as specified in, for example, claim 1, is clearly distinguished from the controlled methylation described in Herman. Thus, those skilled in the art readily recognize that invention methods detect nucleic acid which exhibits aberrant methylation of CpG (thereby indicating the presence of a hepatic cell proliferative disorder) rather than the controlled methylation associated with ordinary biological function.

Applicants further disagree with the Examiner's assertion (see Office Action mailed April 23, 2002, page 8, lines 6-12) that the specification allegedly provides no guidance regarding the detection of methylated CpG-containing GST nucleic acid in any biological fluid. Those skilled in the art recognize that inappropriately proliferating cells (e.g., cancer cells) are invasive, i.e., these cells have the ability to spread to several different biological tissues. Thus, as set forth in the specification (see page 15, line 28 to page 16, line 2) the nucleic acid-containing specimen used for detection of methylated CpG may be from any tissue source, including, for example, colon, blood, lymphatic, hepatic, and the like.

Applicants further disagree with the Examiner's assertion (see Office Action mailed April 23, 2002, page 10, lines 9-15) that the specification allegedly has not established an association between a hepatic cell proliferative disorder and detection of methylated CpG-containing GST nucleic acid in any subject. In efforts to support this assertion, the Examiner alleges that the art is

unpredictable since the specification teaches that GSTP1 mRNA was absent in human Hep3B cells, while Imai, et. al. (*Carcinogenesis*, 18, p 545-551, 1997) teach that expression of GST-P mRNA was high in rat hepatocellular carcinoma samples. This issue is respectfully submitted to be irrelevant. The present invention is directed toward methods for detecting a hepatic cell proliferative disorder comprising detecting a methylated CpG-containing GST nucleic acid. The level of GST-P mRNA present in a specimen has no bearing on the detection of methylated CpG-containing GST nucleic acid.

Applicants also disagree with the Examiner's assertion (see Office Action mailed April 23, 2002, page 11, lines 1-9) that the specification allegedly has not established that a hepatic cell proliferative disorder can be detected by detecting a decreased level of GST RNA in comparison to GST RNA in normal cells. As set forth in the specification, cancerous human liver cells fail to express GST mRNA (see, e.g., page 6, lines 27-28 ; page 35, line 20 to page 36 line 31). In particular, the specification discloses that human hep3B HCC cells failed to express GSTP1 mRNA. Since detecting and comparing levels of GST RNA in cells merely requires routine analysis well within the capability of those skilled in the art, it is respectfully submitted that undue experimentation is not required to detect a hepatic cell proliferative disorder by detecting a decreased level of GST RNA in comparison to GST RNA in normal cells

Applicants' further disagree with the Examiner's assertion (see Office Action mailed April 23, 2002, page 11, lines 1-9) that the specification allegedly does not teach an association between hepatocellular carcinoma (HCC) and hypermethylation in any region of the nucleic acid encoding GST. The present specification teaches that there is a positive correlation between CpG island hypermethylation and HCC (see, e.g., page 8, lines 19-28). This finding is clearly supported by the Examples provided in the specification. Although the invention is illustrated with reference to CpG island hypermethylation in the promoter region of nucleic acid encoding GST, the Examiner has provided no evidence to call into question Applicants' claim that CpG island hypermethylation in any region of a nucleic acid encoding GST positively correlates with hepatocarcinogenesis. Contrary to the Examiner's assertion, undue experimentation is not

required to determine the level of hypermethylation in any region of a nucleic acid encoding GST. Instead, those skilled in the art are only required to perform routine analyses to determine the level of CpG island hypermethylation in any region of the polynucleotide.

For all of the reasons set forth above, it is respectfully submitted that the entire scope and breadth of the claims is fully enabled. Accordingly, reconsideration and withdrawal of the rejection of claims 1-51, 84 and 85 under 35 U.S.C. 112, first paragraph are respectfully requested.

CONCLUSION

In view of the above amendments and remarks, reconsideration and favorable action on all claims are respectfully requested. In the event any matters remain to be resolved, the Examiner is requested to contact the undersigned at the telephone number given below so that a prompt disposition of this application can be achieved.

Respectfully submitted,

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Enclosures: Exhibits A and B

Exhibit A

VERSION WITH MARKINGS TO SHOW CHANGES MADE

1. (Twice amended) A method for detecting a hepatic cell proliferative disorder in a human, comprising: detecting a methylated CpG-containing glutathione-S-transferase (GST) nucleic acid in a hepatic specimen or biological fluid wherein a methylated GST nucleic acid is indicative of a hepatic cell proliferative disorder.

3. (Amended) The method of claim 2, wherein the primers flank a region in the promoter of GST, wherein said promoter contains a transcriptional start site for GST.

4. (Twice amended) The method of claim 3, wherein the promoter region is approximately -539 to -239 upstream from the transcriptional start site.

6. (Amended) The method of claim 1, wherein the detecting comprises contacting a nucleic acid-containing hepatic specimen or biological fluid with an agent that modifies unmethylated cytosine, amplifying the CpG-containing nucleic acid in the specimen by means of CpG-specific oligonucleotide primers, wherein the oligonucleotide primers distinguish between modified methylated and nonmethylated nucleic acid, and
detecting the methylated CpG-containing GST nucleic acid based on the presence or absence of amplification products produced in said amplifying step.

8. (Amended) The method of claim 6, wherein the oligonucleitide primers have a sequence [as set forth in] selected from the group consisting of SEQ ID NO: 7, 8, 9, 10, 11, 12, or 13.

11. (Amended) The method of claim 1, wherein the CpG-containing GST nucleic acid is a promoter region.

16. (Amended) The method of claim 1, wherein the detecting is by contacting a target nucleic acid in the hepatic specimen or biological fluid with a reagent which detects methylation of the promoter region of the GST nucleic acid when the target nucleic acid is DNA, or wherein the reagent detects the level of GST RNA when the target nucleic acid is RNA; and
detecting the GST [target] nucleic acid, wherein hypermethylation of the promoter of GST DNA, or decreased levels of GST RNA, as compared with the level of GST RNA in a normal cell, is indicative of a GST-associated cell proliferative disorder in hepatic tissue.

18. (Amended) The method of claim 1, wherein the GST nucleic acid is a π family GST nucleic acid.

20. (Amended) The method of claim 16 wherein the reagent which detects methylation of the promoter region of the GST nucleic acid is a restriction endonuclease.

27. (Amended) The method of claim 1, further comprising comparing the methylation status of the GST nucleic acid to the methylation status of the GST nucleic acid in adjacent normal hepatic tissue.

39. (Amended) The method of claim 28, further comprising comparing the methylation status of the GST nucleic acid to the methylation status of the GST nucleic acid in adjacent normal hepatic tissue.

41. (Amended) The method of claim 40, wherein the GST nucleic acid is DNA.

42. (Amended) The method of claim 40 wherein the GST nucleic acid is RNA.

51. (Amended) The method of claim 40, further comprising comparing the methylation status of the GST nucleic acid to the methylation status of the GST nucleic acid in adjacent normal hepatic tissue.

Exhibit B

CLAIMS UPON ENTRY OF THE AMENDMENT

1. A method for detecting a hepatic cell proliferative disorder in a human, comprising: detecting a methylated CpG-containing glutathione-S-transferase (GST) nucleic acid in a hepatic specimen or biological fluid wherein a methylated GST nucleic acid is indicative of a hepatic cell proliferative disorder.

2. The method of claim 1, wherein the GST nucleic acid is detected by contacting the nucleic acid with nucleic acid primers.

3. The method of claim 2, wherein the primers flank a region in the promoter of GST, wherein said promoter contains a transcriptional start site for GST.

4. The method of claim 3, wherein the promoter region is approximately –539 to –239 upstream from the transcriptional start site.

5. The method of claim 2, wherein the nucleic acid primers are selected from the group consisting of SEQ ID NO:1, 2, 7, 8, 9, 10, 11, 12, 13, and combinations thereof.

6. The method of claim 1, wherein the detecting comprises contacting a nucleic acid-containing hepatic specimen or biological fluid with an agent that modifies unmethylated cytosineamplifying the CpG-containing nucleic acid in the specimen by means of CpG-specific oligonucleotide primers, wherein the oligonucleotide primers distinguish between modified methylated and nonmethylated nucleic acid, and detecting the methylated CpG-containing GST nucleic acid based on the presence or absence of amplification products produced in said amplifying step.

7. The method of claim 6, wherein the amplifying step is the polymerase chain reaction (PCR).

8. The method of claim 6, wherein the oligonucleotide primers have a sequence selected from the group consisting of SEQ ID NO: 7, 8, 9, 10, 11, 12, and 13.

9. The method of claim 6, wherein the modifying agent is bisulfite.

10. The method of claim 6, wherein cytosine is modified to uracil.

11. The method of claim 1, wherein the CpG-containing GST nucleic acid is a promoter region.

12. The method of claim 1, wherein the specimen is from a hepatic tissue.

13. The method of claim 1, wherein the biological fluid is bile or blood.

14. The method of claim 6, further comprising contacting the nucleic acid with a methylation sensitive restriction endonuclease.

15. The method of claim 14, wherein the restriction endonuclease is selected from the group consisting of *MspI*, *HpaII*, *BssHII*, *BstUI* and *NotI*.

16. The method of claim 1, wherein the detecting is by contacting a target nucleic acid in the hepatic specimen or biological fluid with a reagent which detects methylation of the promoter region of the GST nucleic acid when the target nucleic acid is DNA, or wherein the reagent detects the level of GST RNA when the target nucleic acid is RNA; and
detecting the GST nucleic acid, wherein hypermethylation of the promoter of GST DNA, or decreased levels of GST RNA, as compared with the level of GST RNA in a normal cell, is indicative of a GST-associated cell proliferative disorder in hepatic tissue.

17. The method of claim 16, wherein the reagent is a nucleic acid primer selected from the group consisting of SEQ ID NO:1, 2, 7, 8, 9, 10, 11, 12, 13, and combinations thereof.

18. The method of claim 1, wherein the GST nucleic acid is a π family GST nucleic acid.

19. The method of claim 18, wherein the π family GST is GSTP1.

20. The method of claim 16 wherein the reagent which detects methylation of the promoter region of the GST nucleic acid is a restriction endonuclease.

21. The method of claim 20, wherein the restriction endonuclease is methylation sensitive.

22. The method of claim 21, wherein the restriction endonuclease is selected from the group consisting of *MspI*, *HpaII*, *BssHII*, *BstUI* and *NotI*.

23. The method of claim 16, wherein the reagent is a nucleic acid probe.

24. The method of claim 23, wherein the probe is detectably labeled.

25. The method of claim 24, wherein the label is selected from the group consisting of a radioisotope, a bioluminescent compound, a chemiluminescent compound, a fluorescent compound, a metal chelate, and an enzyme.

26. The method of claim 1, further comprising detecting the presence of hepatitis B virus or hepatitis C virus.

27. The method of claim 1, further comprising comparing the methylation status of the GST nucleic acid to the methylation status of the GST nucleic acid in adjacent normal hepatic tissue.

28. A method for detecting a hepatic cell proliferative disorder associated with a glutathione-S-transferase (GST) in a subject comprising:

contacting a target nucleic acid in a sample of hepatic tissue or biological fluid from the subject with a reagent which detects the GST, wherein the reagent detects methylation of the promoter region of the GST when the target nucleic acid is DNA, and wherein the reagent detects the level of GST RNA when the target nucleic acid is RNA; and

detecting the GST target nucleic acid, wherein hypermethylation of the promoter of GST DNA, or decreased levels of GST RNA, as compared with the level of GST RNA in a normal cell, is indicative of a GST-associated cell proliferative disorder in hepatic tissue.

29. The method of claim 28, wherein the GST is a π family GST.

30. The method of claim 29, wherein the π family GST is GSTP1.

31. The method of claim 28, wherein the biological fluid is bile or blood.

32. The method of claim 28, wherein the reagent which detects methylation of the promoter region of the GST is a restriction endonuclease.

33. The method of claim 32, wherein the restriction endonuclease is methylation sensitive.

34. The method of claim 33, wherein the restriction endonuclease is selected from the group consisting of *MspI*, *HpaII*, *BssHII*, *BstUI* and *NotI*.

35. The method of claim 28, wherein the reagent is a nucleic acid probe.

36. The method of claim 35, wherein the probe is detectably labeled.

37. The method of claim 36, wherein the label is selected from the group consisting of a radioisotope, a bioluminescent compound, a chemiluminescent compound, a fluorescent compound, a metal chelate, and an enzyme.

38. The method of claim 28, further comprising detecting the presence of hepatitis B virus or hepatitis C virus.

39. The method of claim 28, further comprising comparing the methylation status of the GST nucleic acid to the methylation status of the GST nucleic acid in adjacent normal hepatic tissue.

40. A method for detecting a hepatic cell proliferative disorder associated with a glutathione-S-transferase (GST) nucleic acid in a subject, comprising contacting a target cellular component containing a GST nucleic acid with a reagent which reacts with the GST nucleic acid and detecting hypermethylation of the GST nucleic acid, wherein hypermethylation of the GST nucleic acid is indicative of a hepatic cell proliferative disorder.

41. The method of claim 40, wherein the GST nucleic acid is DNA.

42. The method of claim 40, wherein the GST nucleic acid is RNA.

43. The method of claim 40, wherein the reagent is a probe.

44. The method of claim 43, wherein the probe is nucleic acid.

45. The method of claim 43, wherein the probe is detectably labeled.

46. The method of claim 45, wherein the label is selected from the group consisting of a radioisotope, a bioluminescent compound, a chemiluminescent compound, a fluorescent compound, a metal chelate, or an enzyme.

47. The method of claim 40, wherein the reagent is a restriction endonuclease.

48. The method of claim 47, wherein the restriction endonuclease is methylation sensitive.

49. The method of claim 48, wherein the restriction endonuclease is selected from the group consisting of *MspI*, *HpaII*, *BssHII*, *BstUI* and *NotI*.

50. The method of claim 40, further comprising detecting the presence of hepatitis B virus or hepatitis C virus.

51. The method of claim 40, further comprising comparing the methylation status of the GST nucleic acid to the methylation status of the GST nucleic acid in adjacent normal hepatic tissue.

84. The method as in any of claims 1, 28, or 40, wherein methylation is in one allele.

85. The method as in any of claims 1, 28, or 40, wherein methylation is in both alleles.